HIGHLIGHTS FROM RECENT LITERATURE

THE EXPANDING ROLE OF ENDOGENOUS RNAs IN AUTOIMMUNE INFLAMMATION

Review of:

Systemic lupus erythematosus (SLE) is often associated with the presence of autoantibodies such as anti-nuclear antibodies (ANA), elevated levels of serum cytokines including type I interferons (IFNs) and IFN-inducible transcripts known collectively as the “IFN signature” detected in peripheral blood leukocytes and target organs. Mavragani et al. explored the hypothesis that RNAs generated from endogenous repetitive genomic sequences, which constitute a substantial fraction of the human genome, might serve as ligands for nucleic acid sensors triggering type I IFN production in SLE. Mammalian retrotransposons, ancient endogenous retroviruses that have integrated into the genome, are composed of three families including the long interspersed nuclear element (LINE) family, with as many as 500,000 copies per haploid genome, the more well-known Alu elements, with an estimated 500,000 to 1,000,000 copies per human genome, and the SVA family, composed of consensus sequences of Short Interspersed Elements (SINEs), Variable Number of Tandem Repeats (VNTR) sequences and Alu elements, with 3,700 sequences per human genome (PMID: 23523050).

LINE1 represents the only known active autonomous mobile retrotransposon in humans since Alu and other elements are dependent on LINE1 for mobilization. Few full-length transcripts of LINE1 have been found in the human genome as the majority of LINE1 sequences have been truncated or mutated and are not able to transpose, but inserted elements can interfere with genome function. LINE1 RNA expression is repressed by methylation of CpG islands in the promoter region. Thus, altered regulation of genome methylation likely provides a major mechanism for LINE1 mRNA expression. LINE1 encodes 2 proteins from 2 open reading frames (ORFs), ORF-1/p40 and ORF-2, about 150 kd. The function of ORF-1 is unknown, however in the cytoplasm it colocalizes with LINE1 transcripts in RNP particles, whereas ORF-2 has endonuclease and reverse transcriptase activity.

Findings:

- Mavragani et al. demonstrated that renal biopsy samples from SLE patients with lupus nephritis class III, class IV and class V (membranous nephritis) had elevated LINE1 full-length mRNA levels as compared to healthy control samples. Elevated levels of LINE1 transcripts were detected in minor salivary gland (MSG) tissue from primary
SS, autoimmune sicca syndrome and other autoimmune diseases as compared to similar samples from non-autoimmune sicca controls or pooled healthy donors.

- Immunohistochemistry revealed the presence of ORF-1 protein in renal tubular cells of lupus nephritis renal biopsies, in ductal epithelial cells from SS MSG tissues and in inflammatory infiltrates. Serial samples demonstrated the presence of IFNβ expressed by the same cells in these tissues along with IFNα expressed by BDCA-2+ PDC within the inflammatory infiltrates. ORF-1 protein expression in autoimmune tissues was confirmed by western blots.

- LINE1 transcript levels correlated with type I IFN transcript levels in SS MSG tissues and in lupus nephritis renal samples, albeit to a lesser degree. Thus, LINE1 transcripts could potentially drive TLR-dependent and/or TLR-independent type I IFN production.

- A negative correlation was observed between LINE1 mRNA levels and percent of LINE1 methylation for most LINE1 CpG promoter sites evaluated in DNA samples from SS MSG tissue as compared to age-matched sicca control tissue. Thus, the data are consistent with LINE1 promoter demethylation as a mechanism for LINE1 expression.

- Type I IFNs were unable to induce LINE1 transcripts in in vitro experiments. No LINE1 transcripts were detected in (a) peripheral blood mononuclear cells from healthy ones, (b) the human embryonic kidney line, HEK 293F and (c) non-cancerous patient salivary epithelial cells stimulated with human recombinant IFNα or agonists to TLR7 and TLR9, whereas IFN-inducible genes, such as IFIT1 and IFIT3, were upregulated in a dose-dependent fashion.

- Human PDC and freshly isolated CD14+ monocytes transfected with a plasmid containing the promoter and full-length LINE1 retrotransposon (cloned from an SLE patient's X chromosome) but not empty vector control cells produced increased levels of IFN2α mRNA. Moreover, PDC and monocytes transfected with the 5’ untranslated region (UTR) mRNA from LINE1 and U1 RNA (related to Sm/RNP) potently induced IFNα mRNA and IFN bioactivity was detected in culture supernatants. Control RNA and hY3 RNA (related to Ro RNP) lacked these potent immunostimulatory activities. Thus, specific RNA secondary structures of the 5’ UTR LINE1 and U1 RNAs might be required for LINE1 RNA activation of nucleic acid sensors and IFN production.

- Both TLR-dependent and TLR-independent pathways contributed to the 5’ UTR LINE1 immunostimulatory capacity as type I IFN production was significantly reduced by specific inhibitors, the TLR7/8 inhibitor IRS 661 in PDCs and inhibitor Bx795 in monocytes. Bx795 acts downstream of nucleic acid sensors to inhibit IKKe/TANK-binding kinase 1 (TBK1).

The study by Mavragani et al. demonstrated that LINE1 transcripts and protein were expressed in target tissues obtained from autoimmune patients and correlated with elevated type I IFN production. Furthermore, LINE1 RNA stimulated nucleic acid sensors and induced type I IFNs in in vitro experiments. Expression of endogenous retrotransposable elements such as LINE1 are commonly observed in various cancers (PMID: 23555307) and elevated LINE1 expression has been observed in lupus models (PMID: 12165858) and human rheumatoid synovial tissue (PMID: 11145021). Increased LINE1 transcripts can be induced by hypomethylation of promoter CpG motifs in affected tissues suggesting a potentially important contribution of epigenetic alterations in modifying inflammatory responses. An increased understanding of the mechanisms involved in the regulation of retrotransposon expression could provide new targets for therapy.

*Reviewed by Laurie Davis, PhD, UT Southwestern Medical Center*
WORMING OUR WAY OUT OF ASTHMA

Research Article:

Allergic and autoimmune disorders such as asthma have been increasing in prevalence in the developing world in parallel with the decreasing prevalence of parasitic infections. Experimental hookworm infections are currently showing promise in clinical trials for their ability to suppress inflammatory diseases, such as multiple sclerosis, asthma, and inflammatory bowel disease. Clearly, parasitic worm infections produce immunomodulatory factors that can help to decrease inflammatory responses. However, infecting patients with a live pathogen has obvious drawbacks. In this study, the authors identify a promising anti-inflammatory factor produced by hookworms and characterize its immunologic effects.

- The authors studied a protein secreted by hookworms called inflammatory protein-2 (AIP-2). They synthesized a recombinant version of AIP-2 and found that it was capable of protecting against inflammation in an ovalbumin-induced mouse model of allergic airway disease. Administration of AIP-2 by inhalation was also effective in preventing and treating airway inflammation.
- The authors found that CD103+CD11c+ dendritic cells in the mesenteric lymph nodes of these mice were the main population to capture AIP-2 and that these cells downregulated MHC II after AIP-2 exposure. CD103+CD11c+ dendritic cells in mesenteric lymph nodes have been shown to be highly tolerogenic in other systems. Depletion of these cells prevented the anti-inflammatory effect of AIP-2.
- AIP-2 treatment was also found to double the number of immunosuppressive regulatory T cells (Tregs) in the airway and intestinal mucosa. Co-cultures of T cells with mesenteric lymph node CD103+CD11c+ dendritic cells from AIP-2 treated mice led to increased Treg generation. Removal of the mesenteric lymph nodes prevented the increase in airway and G.I. mucosal Tregs, suggesting that the mesenteric lymph node environment was critical for the differentiation of gut homing Tregs. Moreover, AIP-2 treatment induced a long-term tolerogenic “imprint” in the mesenteric lymph nodes that provided long-lasting generation of Tregs.
- Peripheral blood cells from human subjects with clinically characterized human dust mite allergy showed less T cell proliferation when stimulated in the presence of antigen and AIP-2.

The authors have studied an anti-inflammatory protein produced by hookworms, generated the recombinant protein and demonstrated that it has potent effects on suppressing airway inflammation. Many of these effects are mediated by the expansion of a highly tolerogenic population of dendritic cells that can in turn generate large numbers of regulatory T cells that home to the mucosal tissues of the gut and lung. These dendritic cells appear to persist long-term in mesenteric lymph nodes, providing long-lasting tolerogenic signals to T cells. This promising anti-inflammatory protein may have the potential to improve treatment of asthma and other mucosal based inflammatory diseases, such as inflammatory bowel disease and celiac disease.

Reviewed by Rachael A. Clark, MD, PhD, Brigham and Women's Hospital
EARLY DYSREGULATION LEADS TO AUTOIMMUNE DISEASE IN ADULTS

Research Paper:

Antibodies against self-proteins can be present for years to decades before the development of overt autoimmune disease, suggesting that a loss of tolerance may exist amongst circulating immune cells that precedes tissue injury. The authors studied autoimmunity in a mouse model of Sjögren’s disease in order to identify the type and timing of immune dysregulation that made these mice susceptible to inflammatory disease later in life.

- Sjögren’s disease is a chronic autoimmune disorder that involves immune destruction of the salivary and tear glands, leading to destructive dryness of the mouth and eyes. Immune cells that inflame the salary gland often organize into tertiary lymphoid structures, containing germinal center-like areas where follicular dendritic cells (FDC) and autoreactive B cells cluster together, leading to production of destructive autoantibodies.

- In animal models, initiation and maintenance of germinal centers requires CD40, a member of the tumor necrosis factor receptor superfamily. CD40 is expressed in tertiary lymphoid like structures in the salivary glands in both humans with Sjogren’s disease and in Sjogren’s mouse models.

- The authors focused on NOD.H-2h4 mice, a strain that spontaneously develops autoantibodies at a young age, followed later by inflammation and destruction of the salivary glands, leading to dry mouth.

- At a young age, these mice formed spontaneous germinal centers in the spleen that coincided with autoantibody production and preceded salivary gland inflammation. CD40 signaling was required for the formation of these germinal centers.

- A single dose of CD40 ligand (CD40L) early in life, an agent that disrupts CD40 signaling, abolished the formation of salivary gland tertiary lymphoid structures and prevented the mice from eventually developing the disease. Splenectomy had the same effect.

- A single prophylactic treatment with anti-CD40L also blocked the development of autoimmune thyroiditis and diabetes in susceptible mouse strains.

The authors have shown that immune dysregulation occurs early in life in the spleen and that this dysregulation precedes the development of tissue inflammation in mouse models of Sjogren’s disease. A single dose of CD40L reversed this dysregulation and prevented the development of autoimmune disease later in life. This work is notable because it shows that immune dysregulation in the spleen precedes the development of autoimmune disease and if this early dysregulation can be blocked using CD40L, the later development of autoimmune disease can be prevented. Further studies in humans are needed to determine if similar events occur in the spleen before and after the emergence of overt autoimmune diseases. If so, safe and effective blockade of CD40 signaling in patients with autoantibodies could prove to be an effective way to prevent the eventual development of autoimmune disorders.

Reviewed by Rachael A. Clark, MD, PhD, Brigham and Women’s Hospital
EVIDENCE FOR T CELL MEDIATED IMMUNOEEDITING IN HUMAN TUMORS

Research Paper:

Specific recognition of tumor associated neo-antigens by tumor infiltrating T cells is well recognized as an important component of anti-tumor immunity. This is particularly true in cancers with high mutational burdens, such as melanoma or mismatch repair deficient colorectal adenocarcinoma, where the neo-antigen burden appears to correlate not only with influx of neo-antigen specific T cells but also with responses to immune checkpoint blockade (1-3). Understanding the repertoire of neo-antigens expressed in a particular tumor or tumor-type will have significant implications for the rational application of immunotherapy in the future.

Despite their importance for anti-tumor immunity, the temporal dynamics and stability of neo-antigen expression in human cancer remains poorly understood. According to the concept of cancer immunoediting, T cell responses to neo-antigens will provide initial control of a nascent tumor, but will eventually result in immune escape by selecting for tumor clones that lack expression of highly immunogenic peptides. Although this concept has been elegantly demonstrated in murine tumor models, its relevance to human cancer and responsiveness to immunotherapy has been uncertain (4-6).

To address these questions, Verdegaal et al. assessed temporal changes in neo-antigen expression and neo-antigen recognition by autologous tumor-specific T cells in two patients with metastatic melanoma. The authors studied the two patients over an extended follow-up period spanning over 10 years. Both patients had cell lines established at baseline and during disease progression from surgically resected metastatic tumors. These cell lines were used to stimulate peripheral blood mononuclear cell fractions in order to generate tumor specific autologous T cells, which were then infused back into the patients at various clinical time-points. T cell responses to specific neo-antigens were mapped by loading autologous antigen-presenting cells with a comprehensive panel of synthetic 31-mer peptides that were designed to encompass the entire landscape of somatic, non-synonymous coding mutations present in the individual patient’s tumor samples and corresponding cell lines.

Using these techniques, the study authors made some of the following observations:

- Autologous tumor specific T cells reacted primarily to private epitopes that were specific to the patient’s individual tumor, rather than common epitopes shared across tumors derived from different patients (including from HLA-matched individuals).
- Patient one had demonstrable cytotoxic T cell responses to two specific neo-antigens in a baseline tumor sample: KIAA0020 (P451L), which encodes the minor histocompatibility antigen HA-8, and RPL28 (S76F), which encodes the ribosomal protein L28.
- Patient one showed loss of the RPL28 (S76F) variant in a subsequent brain metastasis that was biopsied following disease progression (but prior to administration of autologous T cell therapy); a cell line derived from this brain metastasis provoked less of a cytotoxic T cell response relative to a cell line derived from the baseline sample, which contained the intact RPL28 (S76F) variant.
- Patient one experienced a complete response to the autologous T cell therapy, which has reportedly been maintained for over 9 years and is ongoing.
Patient two had demonstrable cytotoxic T cell responses to three specific neo-antigens in a baseline tumor sample: EML1 (R64W), which encodes the Echinoderm Microtubule Associated Protein Like 1; SEPT2 (R300C), which encodes the Septin 2 protein; and CAD (R1854Q), which encodes the Carbamoyl-Phosphate Synthetase 2, Aspartate Transcarbamylase, And Dihydroorotase protein.

Patient two showed decreased tumor infiltrating T cell responses against the SEPT2 (R300C) and CAD (R1854Q) epitopes in metastatic tumor deposits that developed six years after the administration of autologous T cell therapy; interestingly, these findings were coincident with a 10-fold reduction in SEPT2 RNA expression (but preservation of the SEPT2 (R300C) DNA variant) in addition to loss of the CAD (R1854Q) DNA variant (but maintenance of total CAD RNA expression).

Patient two died approximately 11 years after diagnosis and experienced a 4-year disease free period following autologous T cell therapy.

All neo-antigens that provoked cytotoxic T cell responses were not present within the Catalogue of Somatic Mutations in Cancer (COSMIC) database, with the exception of SEPT2 (R300C), which appears once; this implies that the neo-antigenic mutations are “passenger” alterations without a direct impact on tumorigenesis.

Although the conclusions to be drawn are limited by the small number of patients included in the study, the authors show preliminary evidence that immunoediting occurs in human tumors. These findings imply that the set of potentially targetable neo-antigens in a tumor may change dynamically with time or changes in clinical and/or treatment status. As the authors rightly conclude, immunotherapy efforts would be well served exploit the adaptive capacity of the immune system whenever possible in order to accommodate the range of potential neo-antigens that may emerge during a patient’s clinical course.

References


Reviewed by Gabriel Griffin, MD, Brigham and Women’s Hospital
**ARE HUMAN ILC’S DISPENSABLE?**

**Research Paper:**


Innate lymphoid cells (ILCs) have recently become the focus of intense scrutiny. They include three subsets (ILC1, ILC2, ILC3) that resemble CD4+ helper T cell subsets (but lack TCR expression) as well as NK cells. The details of their ontogeny are more clear in mouse models than in human data at this time. This study examines ILCs in patients with severe combined immune deficiency (SCID) caused by two mutations that affect both T and NK cells (sparing B cells): JAK3 and IL2RG SCID. This was chosen as ILCs are known to require IL-7, which signals via the IL2RG and JAK3 pathways, thus allowing assessment of ILC deficiency as a component within SCID.

**SUMMARY**

- They begin by examining ILC frequency in peripheral blood of healthy patients (both pediatric and adult). Interestingly, helper-T cell like ILC decrease somewhat with age, and overall counts of ILCs are very low (3.8x10^3/ml in children and 1.6x10^3/ml in adults), while. Relative frequencies of ILC subsets did not vary with age.
- They next examined ILC subsets in 28 SCID patients (12 IL2RG deficient 9 JAK3 deficient and 7 deficient in RAG1 or RAG2; the latter as comparators, along with 6 healthy controls).
- They had access to pre-hematopoetic stem cell transplantation (HSCT) samples in 5 and post-HSCT samples in 20.
  - As expected, in the 5 patients with samples prior to HSCT: no ILCs were found in the 3 JAK3 patients, while ILCs were normal in RAG SCID.
  - They also studied 18 patients with JAK3 or IL2RG SCID who had already had HSCT prior to the study sampling, each had received mild or non-myeloablative conditioning (MAC) and 6 RAG1 or RAG2 SCID patients who received myeloablative conditioning (MAC) prior to HSCT. While both patient populations had appropriate T cell reconstitution.
    - Those who had not had MAC (and in this study, these patients had JAK3 or IL2RG SCID), had partial chimerism wherein only T cell were from the donor.
    - However, those who had MAC (and in this study, were RAG1 or RAG2 SCID) had complete donor chimerism.
    - NK and ILC1s were generally not found in peripheral blood or skin and gut tissue samples from JAK3 or IL2RG SCID patients post-HSCT (though ILC1s were in one adult and one pediatric patient in peripheral blood).
    - ILC2s and ILC3s were also not found in the tissue sections from JAK3 or IL2RG SCID patients post-HSCT with non-MAC.
      - Of note, ILC reconstitution was also not seen in the single patient with IL2RG SCID who received MAC pre HSCT, but partial tissue ILC reconstitution was seen in a SCID patients who received mild MAC.
    - After HSCT for RAG SCID or a set of hematological oncological processes (both received MAC prior to HSCT), complete donor chimerism for NK cells and ILC1-3 was seen.
- They also examined ILC reconstitution in murine HSCT, using a sublethally irradiated (or non-irradiated) Rag2/-/ Il2rg/-/ recipient mice with reconstitution of ILC2 and ILC3s in sublethally irradiated recipients but not in non-irradiated recipients. ILC1s were more easily reconstituted in non-irradiated mice.
Finally, they note that the persistent lack of ILC post-HSCT in JAK3 and IL2RG SCID patients was not correlated with a compensatory increase in other innate cells (e.g. γδT cells and iNKT cells) or an increase in infections beyond the baseline increase in HPV in these forms of SCID post-HSCT.

Studying patients who have deficiencies in T, NK and ILC populations due to genetic mutations and then following their clinical status post-HSCT allows investigation of the effects of isolated NK and ILC absence, which is fascinating. A number of important questions remain, many focused on the long-term effects of lack of NK cells and ILCs in these patients, both on infections as well as malignancy and autoimmune predispositions, given that these patients are generally healthy, but are also still quite young. As we have not yet identified patients who lack ILCs alone, this type of investigation will allow deeper investigation of the roles of this complex new set of immune cells within the human immune system, with important paired studies with mouse models.

Reviewed by Sarah Henrickson, MD, PhD, Children’s Hospital of Philadelphia

ALLERGIC TO THE DETAILS? USING RNASEQ TO STUDY DIFFERENCES AMONG TH2 CELLS IN ALLERGIC RHINITIS, ASTHMA AND HEALTHY CONTROLS

Research Paper:

It has become increasingly clear over the last 10 years that asthma is not a monolithic disease with a single causative mechanism and a single optimal treatment plan. However, in many patients, particularly patients with atopy, there can be a clear role for Th2 cells. This study examines the difference in the transcriptome in CD4+ CCR4+ (Th2 enriched) T cells from patients with allergic rhinitis (AR) (without asthma, n = 25), atopic patients with asthma (n = 37 total, 12 on inhaled steroids and 25 using albuterol as needed) and healthy controls without atopy.

SUMMARY

- They sorted human CD4+ CCR4+ T cells and performed RNASeq from 80 samples (77 patients). Using weighted gene coexpression analysis (WGCNA, based on hierarchical clustering) they found 15 gene modules, of which 2 were upregulated and three were downregulated in asthmatics versus healthy controls (of those, four were also significantly different between AR and atopic asthmatics). A similar set of genes was identified using a different analysis strategy, DESeq2.
- They initially on genes identified as differentially expressed in asthma versus HC using DESeq2, and noted that many genes involved in apoptosis, NFkB and TNF signaling, among others, were upregulated in asthma. These included IL17RB (potentially allowing IL-25 responses) as well as mediators in the NFkB pathway and metabolic enzymes.
  - Interestingly, when the genes that identified asthmatics vs. HC were examined in AR patients, their expression level was noted to be intermediate.
- Next, they focused on genes that were differentially expressed in atopic asthmatics versus patients with allergic rhinitis. (n = 344). Here, they noted that atopic asthmatics had downregulated negative regulators of the MAPK and AP-1 pathways. In addition, atopic asthmatics downregulated EP4 (PGE2 receptor) as well as components of autophagy.
  - The asthma specific genes were then examined for correlation with clinical characteristics, including response to bronchodilation, with some examination of potential correlations.
There were no clear differences in trafficking molecules between AR and atopic asthmatic Th2 cells.

- They also tried to assay the frequency of allergen specific CD4+ Th2 cells using an in vitro model (a 2-week culture in vitro with Timothy Grass peptides); this yielded no difference in Timothy Grass specific Th2 cells.

This study examines the question of how Th2 cells contribute to AR versus asthma (and can be present in HC without atopy). This is an interesting question, in that we see different pathology and clinical outcomes in conditions where this cell type is implicated. However, these are complex conditions with broad clinical phenotypes. One important question is therefore how relevant Th2 cells are across the broad and diverse landscape of asthma; many groups work to define the importance of many CD4+ and CD8+ effector populations (and even asthmatics with +skin prick testing for aeroallegens, as studied here, may have diverse mechanisms beyond Th2). With regard to patient selection, age may be a confound, as the AR patients seem to be older than the asthmatics (and children are excluded). In addition, it is unclear how well CCR4 expression provided the desired Th2 enrichment in this protocol. Within the dataset, there are differences in gene expression between each pair of patients (atopic asthmatic, AR and HC), but an analysis of gene signatures that could unite the various interesting individual differences remains to be fully elucidated. Finally, it is unclear how relevant a single allergen (here, Timothy Grass) is to quantify the frequency of allergen specific cells in a patient panel with variable allergen sensitization (though each patient does appear to be Timothy Grass sensitized, most of the patients have multiple sensitizations). While Th2-enriched cells from AR patients versus atopic asthmatics seems by RNASeq in these patient pools to be intermediate in phenotype, having uncovered these potential differences it remains to test these potential hypotheses and by testing the pathways identified as candidates in these patient populations,

Reviewed by Sarah Henrickson, MD, PhD, Children’s Hospital of Philadelphia

DEVELOPMENTS IN BASIC IMMUNOLOGY AND NOVEL THERAPIES

EXPLOITING METABOLISM IN T CELLS TO TREAT IMMUNE MEDIATED DISEASE

Over the previous decade, several groups have elucidated details of metabolic pathways that drive survival, growth and differentiation in T lymphocytes\(^1\,^2\). T cells differ in their utilization of metabolic pathways and therefore energy sources depending on the specifics of T cell subset and state of activation. The bio-energetics needs of a resting naïve lymphocyte are different from one that is rapidly expanding and differentiating or actively regulating immune responses. It is now clear that this influences the quality and immune responses.

Alternate Sources of Energy

Depending on their state of differentiation and activation, T lymphocytes require vastly different amounts of energy and may utilize a variety of fuels\(^2\,^3\). Glucose enters lymphocytes via regulated channels, is trapped in the cell due to rapid phosphorylation of hexokinase and a majority flows through the glycolytic pathway generating pyruvate for the TCA cycle and oxidative phosphorylation. Alternatively, phosphorylated glucose can be used as a carbon source for the lipid and nucleic acid generation that is required in rapidly dividing cells. Both essential and non-essential amino acids must be imported to meet metabolic demands of rapidly dividing lymphocytes. Amino acids such as glutamate and aspartate may also be used as precursors of pyrimidine and purine synthesis. Rapid division similarly requires massive increases in membrane and other lipids which is achieved through a combination of import and \emph{de novo} synthesis.
**T cell Subsets**

Naïve T lymphocytes circulate through blood and lymphatics without the need for cell division and minimal energy requirements. These cells rely on oxidative phosphorylation to generate energy\(^4\). In response to antigenic stimulation in the context of co-stimulation (e.g., TCR and CD28), both CD4 and CD8 T cells undergo rapid clonal expansion and differentiation. Glucose is a major nutrient and enters the cell via T cell receptor and IL-7R signaling induced glucose transporter GLUT1 followed by phosphorylation and entry into the glycolytic pathway. In this case, glycolysis is providing precursors for lipids, nucleic and amino acids. These rapidly proliferating cells can also utilize cellular uptake of the amino acid glutamine as a glycolytic precursor. CD4 T cells may differentiate into Th1, Th2, Th17 or Treg with these populations having overlapping metabolic programs. For example, Th1 and Th2 rely on glucose and glutamate\(^5,6\). Unlike Th1/Th2, cells in the Th17 population rely on hypoxia inducible factor1-alpha (HIF1α)\(^7,8\). Recent work has shown that Treg are dependent phosphatidylinositol-3-OH kinase (PI3K) for lipid oxidation rather than glycolysis\(^9\). The differences in metabolic requirements and pathways may allow for more directed targeting of effector, memory and regulatory subsets to either increase or decrease immune responsiveness depending on the clinical setting.

**Approaches to Modify Immunoregulation Through Metabolism**

There are many publications that have described targeting metabolism in lymphocytes to alter immune function. Due to space limitations, two approaches will be described in greater detail – one that inhibits T cell mediated responses and a second that improves immune responses.

Powell and colleagues have used a combination of compounds that inhibit metabolism at multiple points to prevent experimental allograft rejection\(^10\). 2-deoxyglucose (2-DG) is a glucose analogue that inhibits hexokinase and thus glycolysis; he glutamine analog 6-diazo-5-oxo-L-norleucine (DON) inhibits multiple components of the glutamine metabolic pathway; metformin is a clinically used oral hypoglycemic agent that activates AMP kinase. In combination, the three drugs significantly inhibit effector CD4 and CD8 T cell expansion, antigen-specific IFNγ production, and CTL activity. This treatment also increased the relative frequency of Tregs suggesting differential effects on the regulatory versus effector T cells. Most intriguingly, combination therapy with all three agents led to prolonged acceptance of fully allogeneic vascularized heart or skin transplants in the absence of any additional immunosuppression. Taken together, these data suggest that inhibition of glycolysis and glutamine metabolic pathways, using compounds already used in human patients, may selectively alter T cell populations to promote immunosuppression for autoimmunity and transplantation.

Tyrakis and co-workers recently used a metabolomics approach and identified S-2-hydroxyglutarate (S-2HG) as a key immunometabolite that accumulates in response to hypoxia and T cell receptor crosslinking of CD8^+^ T cells\(^11\). Glutamine is the major source of S-2HG accumulation which is dependent on expression of HIF-1α through lactate dehydrogenase (Ldha). Interestingly, exogenous S-2HG has several effects on CD8 T cell differentiation including increased expression of CD62L, CD127, CD44, 41BB, decreased PD-1 and altered histone methylation. Concomitantly, S-2HG treated CD8 T cells undergo greater homeostatic proliferation, greater in vivo persistence and improved control of tumor growth. Conversely, overexpression of L2hgdh, which oxidizes S-2HG, increases CD8^KLRG1^hi effector cells suggesting decreased memory T cell generation. Taken together, modulation of S-2HG levels offers a potential approach to modify CD8^+^ T cells to vastly alter function.

**References**


Submitted by Jonathan S. Maltzman, MD, PhD, FAST, Veterans Affairs Palo Alto Health Care System and Stanford University

**SELECTED RECENT CLINICAL TRIAL RESULTS**

**ACTIVITY OF SECUKINUMAB, AN ANTI-IL-17A ANTIBODY, ON BRAIN LESIONS IN RRMS: RESULTS FROM A RANDOMIZED, PROOF-OF-CONCEPT STUDY**

**Clinical Trial:** Havrdova E, Belova A, Goloborodko A, et al. *J Neurol* (May 2016) 263:1287-1295

**Disease:** relapsing remitting multiple sclerosis (RRMS)

**Intervention:** secukinumab 10 mg/kg or placebo. Secukinumab is a human monoclonal antibody that binds to IL-17A

**Study design:**
- Double-blind, placebo-controlled study conducted in Czech Republic, Ukraine, Russia.
- 73 subjects aged 18-55 with diagnosis of MS and at least 1 documented relapse within 1 year, 2 documented relapses within 2 years, or positive MRI scan at screening.
- Randomized 1:1 to secukinumab 10 mg/kg iv (n=38) or placebo (n=35) infusions at weeks 0, 2, 4, 8, 12, 16, 20.
- Primary endpoint: cumulative number of combined unique active lesions (CUAL) observed on monthly brain MRI from week 4-24.
- Secondary endpoint: safety/tolerability, relapse, and individual MRI parameters.
- Exploratory endpoint: human beta-defensin-2 (hBD-2) analysis: hBD-2 is a downstream marker of IL-17A activity.

**Results:**
• Primary endpoint: Non-significant 49% reduction of CUAL seen on MRI from week 4 to week 24 in secukinumab group compared to placebo (P=0.087).

• Secondary endpoints:
  o No serious adverse events.
  o Adverse event rate similar in treated (53%) and placebo (49%).
  o Mild-moderate infection more common in treated (37%) compared to placebo (23%).
  o 67% reduction in cumulative new gadolinium enhancing T1 lesions (P=0.003) by week 16.
  o No significant difference in relapse rate at week 24 (P=0.191).

• Exploratory endpoint:
  o Secukinumab treatment was associated with a reduced hBD-2 level at baseline compared to week 4 (P=0.0008); no reduction seen with placebo at baseline compared to week 4 (P=0.20).

Why the Trial is of Interest to the Broader FOCIS community:

The search continues for disease modifying therapies for relapsing remitting MS, the most common type of MS. The IL-12/23 and IL-17 pathways are attractive targets because Th17 cells are implicated in many mouse models of autoimmunity including the mouse model of MS (1). IL-17A is produced by Th17 cells, CD8+ T cells, γδ T cells, astrocytes, and oligodendrocytes in active MS lesions (2-3).

In this proof on concept study, the primary endpoint (the number of CUAL/combined unique active lesions on MRI) at week 24 was not met. However, the number of new gadolinium-enhancing T1 lesions (a secondary endpoint) was reduced by 65% after 16 weeks of secukinumab treatment. Interestingly, when the investigators restricted their analysis to weeks 12-24, the treatment effect was enhanced, which suggests that variability is greater early in treatment and secukinumab may require at least 12 weeks to reduce disease activity as measured by MRI. The investigators did show a significant reduction in hBD2 level, which suggests that the dose used in the study was a saturating dose.

The results of this proof of concept study are interesting to compare with previous unsuccessful MS trials of ustekinumab and briakinumab (4-5), antibodies that target the p40 subunit of both IL-12 and IL-23. As the latter is a critical survival factor for IL-17A-producing CD4+ Th17 cells, this suggests that IL-17A produced by cells other than Th17 is important to MS pathogenesis. Alternatively, the blockade of Th17-mediated inflammation may be more complete with secukinumab than with IL-12/23 p40 blockade. Interestingly, the opposite has been seen in Crohn’s disease treatment, where IL-17A blockade with secukinumab was completely ineffective (6), but IL-12/23 blockade is successful (7). Such dichotomous results suggests mechanistic differences in two diseases thought to be Th17 mediated, and reflect a functional dissociation between the Th17 effector molecule IL-17A and the Th17 survival factor IL-23.

The study had a favorable safety profile, similar to previous studies of secukinumab for psoriasis, rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis (8-11).

There was no difference in relapse rate in this relatively short study; hence, it’s unknown whether a reduction in T1 lesions correlates with clinical improvement or stabilization. Larger studies will explore this question.

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**ANTI-NKG2D MONOClonAL ANTIBODY (NNC0142-0002) IN ACTIVE CROHN’S DISEASE: A RANDOMIZED CONTROLLED TRIAL**

**Clinical Trial:** Allez M, Skolnick B, Wisniewska-Jarosinska et al., Gut 2016 Aug 3. pii: gutjnl-2016-311824.

**Disease:** moderately to severely active Crohn’s disease (CD).

**Intervention:** Anti-NKG2D (NNC0142-0002), an antagonizing human immunoglobulin G4 monoclonal antibody that binds to natural killer group 2 member D (NKG2D) receptor.

**Study design:**
- Multicenter, randomized, double blind placebo controlled: Belgium, Canada, France, Hungary, Israel, Poland, Russian Federation, USA.
- 78 patients ages 18-75, CD ≥ 3 months, Crohn’s Disease Activity Index (CDAI) 220-450 and either CRP ≥ 10 mg/L or endoscopic evidence of inflammation
- 1:1 randomization
- single sc dose of 2 mg/kg anti-NKG2D (n=40) or placebo (n=38)
- primary endpoint: change in CDAI from baseline to week 4
multiple secondary endpoints including:
  - change in CDAI from baseline to week 1, 2, 8 and 12.
  - Proportion of patients in clinical remission (CDAI score < 150) at weeks 1, 2, 4, 12
Significance was defined as p≤ 0.10; two-sided t test.
Futility analysis performed after 74 patients enrolled, based on change in CDAI at 4 weeks
  - Resulted in discontinuation of enrollment after 78 out of planned 100 enrolled.

Results:

- Primary endpoint was not met (change in CDAI score by week 4).
- Key secondary endpoints:
  - Greater change in clinical disease activity scores by week 12 in drug recipients (by 55 points in CDAI, p=0.06, by 2.7 points in Harvey-Bradshaw Index, p=0.01, by 3.46 in physical component of SF-36, p=0.04).
- Significant changes in subgroups:
  - Subgroup of patients who had not previously failed anti-TNF biologic drugs (n=55) had a significantly greater drop in CDAI score on treatment than placebo at weeks 1 (p=0.07), 2 (p=0.05), 4 (p=0.01), 8 (p=0.02), and 12 (p=0.03), corresponding to a greater percent of treated patients having clinical response and remission at multiple time points.
  - Patients with a baseline fecal calprotectin (stool test for intestinal inflammation) level > 250 ug/g (n= 51) had a greater change in CDAI score on treatment than placebo at week 8 (by 66 points, p=0.026) and week 12 (by 108 points, p=0.006).
  - Patients with a baseline CRP ≥ 10 mg/L (n=48) demonstrated a decrease in CRP at week 12 on treatment but not placebo.
  - Patients with baseline CDAI ≥330 (n=37) with higher serum anti-NKG2D concentrations in weeks 0-4 showed larger changes in CDAI at week 4.
- Adverse event (AE) frequencies similar in treatment groups: 73% in anti-NKG2D group; 71% of placebo treated
- Most AEs were mild (49%) or moderate (43%). None of the severe AEs (8%) or SAEs were judged to be study drug related.

Why the Trial is of Interest to the Broader FOCIS community:

Although negative for its primary endpoint, subgroup analysis suggested potential efficacy of NKG2D blockade in cohorts with objective evidence of inflammation (elevated fecal calprotectin) or no prior anti-TNF failure, thus supporting the idea that NKG2D+ cells have an important role in Crohn’s disease pathogenesis. NKG2D is increased on the surface of several proinflammatory T cell subsets, including most CD8+ T cells and NK cells, and a subset of CD4+ T cells which are expanded in Crohn’s disease intestinal mucosa and display a terminally differentiated phenotype. Activation of NKG2D triggers cellular proliferation, cytokine production and cell killing, and costimulation of TCR and NKG2D receptor enhances production of proinflammatory and cytotoxic cytokines by T cells, including TNF-α, IFN-γ and IL-17 (1-3). In pre-clinical trials, murine anti-NKG2D antibodies attenuated development of murine colitis mediated by CD4+ T cell transfer (4,5). Although the paper included only limited cellular data, related primarily to pharmacodynamics, it will be informative if future studies demonstrate changes in NKG2D expression on specific cell populations which either predict or correlate with response to treatment with anti-NKG2D, and thus implicate NKG2D+ populations in Crohn’s disease.

Also noteworthy was the finding that responses at 12 weeks, the final point of observation, were actually better than at earlier timepoints, despite this trial involving only a single dose of anti-NKG2D at study initiation. Indeed, the
pharmacodynamics data presented in this paper demonstrates that serum drug levels and cellular NKG2D receptor occupancy has largely disappeared by week 12, suggesting that transient NKG2D blockade may potentially effect a durable change in the immune system to reduce Crohn's disease activity. A longer follow up period may have revealed how durable the clinical responses were, and whether ongoing blockade of the NKG2D receptor is necessary.

A larger, 4 mg/kg dose was used in a smaller RA trial preceding this report (6), raising the possibility that the 2 mg/kg dose used in this trial was inadequate to achieve the primary endpoint. However, at week 1, nearly 100% receptor occupancy was seen, which remained on average >90% at week 8, and thus suggests that dosage did not limit this agent’s clinical efficacy. Indeed, half of drug recipients showed a response (drop in CDAI of 100 points) at week 4, so the study’s inability to meet its primary endpoint was more due to a high placebo response rate, perhaps owing to the reliance of this study upon clinical disease activity scores.

This raises the main caveat with this study—that few objective endpoints were measured, such as fecal calprotectin, or radiographic or endoscopic measures of disease severity in the affected bowel. With the CDAI being largely (and the Harvey Bradshaw Index being entirely) based upon subjective reporting of patient symptoms, it is possible that the effect of NKG2D blockade on mucosal inflammation was over or underestimated. Furthermore, with no endoscopies in this trial to obtain histological evaluation of disease activity, there is also no opportunity to directly evaluate the effect of NKG2D blockade on the mucosal immune system. As clinical trials in Crohn’s disease are increasingly requiring endoscopic evaluations to objectively demonstrate efficacy, this limitation hopefully will be addressed with future trials of NKG2D blockade. However, with the officially negative results of this study and the sponsor’s relinquishing development of immune-based therapeutics, it is unclear if or when future trials of this specific agent will occur.

References:

6. NCT 00927927 First-in-man Trial of NNC0142-0002 in Patients with Rheumatoid Arthritis

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